Mechanisms of Action of OPC-28326, a Selective Hindlimb Vasodilator

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ABSTRACT

The unique cardiovascular profile of OPC-28326 [4-([N-methyl-2-phenylethylamino]-1-(3,5-dimethyl-4-propionylaminobenzo-yl)piperidine hydrochloride monohydrate] provides insight into basic mechanisms of this new drug as determined by experiments in dogs and rats. In anesthetized open-chest dogs, an i.v. administration of a low dose (0.3 and 1.0 μg/kg) of OPC-28326 selectively increased femoral artery blood flow with only minimal action on systemic blood pressure, heart rate and coronary, carotid, vertebral, renal, and mesenteric blood flows. Biochemical study suggests that OPC-28326 had no effect on phosphodiesterase-3 and -5. OPC-28326 dose-dependently inhibited phenylephrine-induced increases in blood pressure in spinally anesthetized dogs. The potency of OPC-28326 was, however, about 180 times lower than that of prazosin. Although binding studies have revealed an affinity of OPC-28326 to serotonin 5-HT2 receptors, the drug is without effect, except at very high concentrations, on serotonin-induced contraction in an isolated canine femoral artery preparation. The potency of OPC-28326 on the increase in femoral artery blood flow was about 14 times higher than that of prazosin but was at about the same level as that obtained with yohimbine in canine autoperfused femoral artery preparations. In perfused rat hindlimb preparations, OPC-28326 inhibited the decrease in perfusion flow induced by brimonidine, a selective α2-adrenoceptor agonist. The potency of OPC-28326 was at least 10 times less than that of yohimbine. Taken together, the results show that at low doses, OPC-28326 selectively exerts a potent vasodilating effect on the femoral arterial bed, in part due to an α2-adrenoceptor-blocking activity.

Vasodilators are used in a variety of conditions depending on the specificity of the individual drug. For example, those that primarily dilate peripheral resistance vessels are used as the major treatment modality in hypertension; these drugs include calcium channel antagonists, potassium channel openers, and direct and indirect inhibitors of intrinsic vasoconstriction such as α-adrenergic antagonists, angiotensin receptor antagonists, and angiotensin-converting enzyme inhibitors (van Zwieten, 1993). Calcium antagonists also have relatively selective coronary vasodilator action (Taira, 1987), and a hybrid of nitrate and the ATP-sensitive potassium channel opener nicorandil preferentially dilates coronary arteries (Sakai et al., 1981; Taira, 1989). These are used as antianginal agents. A series of 1,4-dihydropyridines such as nimodipine are relatively selective for cerebral vessels (Freedman and Waters, 1987) and has been used with some success in reducing morbidity rates after a subarachnoid hemorrhage (Allen et al., 1983; Gelmers et al., 1988).

We systematically screened a large number of compounds for coronary and femoral vasodilator actions and identified a quinolinone compound, with a 4-amino-1-piperidinylcarbonyl moiety, that preferentially increased femoral artery blood flow (FBF) but not coronary artery blood flow (CBF). Further optimization of this compound resulted in a lead candidate OPC-28326 [4-([N-methyl-2-phenylethylamino]-1-(3,5-dimethyl-4-propionylaminobenzoyl)piperidine hydrochloride monohydrate; Fig. 1]. This compound has a unique cardiovascular profile in that it appears to be highly selective for the femoral arterial bed.

It was reported that buflomedil (Clissold et al., 1987; Kushiku et al., 1989), TA-993 (Kaburaki et al., 1998), and vintoperol (Szombathelyi et al., 1991) increased FBF. These drugs, however, have many cardiovascular side effects. Buflomedil...
and TA-993, for example, increased blood flow to other organs, such as the brain (Kushiku et al., 1989; Kaburaki et al., 1998). Vintoprenal decreased systemic blood pressure (Szombathy et al., 1991) at the same dose that increased FBF. A drug with a high selectivity for the femoral arterial bed would be of potential importance in the treatment of peripheral arterial insufficiency.

The purpose of the present study was to elucidate information on the mechanism of action of OPC-28326 using a variety of techniques in dogs and rats.

Materials and Methods

Mongrel dogs of either sex and Sprague-Dawley male rats (SLC, Shizuka, Japan) were housed during the experiment in an air-conditioned (temperature-, humidity-, and light-controlled) animal room. All experiments were performed under the regulations of the Guidelines for Animal Experimentation (Otsuka Pharmaceutical Co., Ltd.).

Dogs weighing 8 to 23 kg were anesthetized with 30 mg/kg pentobarbital sodium i.v., and rats weighing 270 to 400 g were anesthetized with 50 mg/kg pentobarbital sodium i.p. All measurements were made with a thermal pen recorder (Recti-Horiz 8K; NEC Medical Systems, Tokyo, Japan).

Cardiovascular Effects of OPC-28326 in Anesthetized Open-Chest Dogs

After induction of anesthesia, an endotracheal cannula was inserted and connected to a respirator (model SN-480-3; Shinnano, Tokyo, Japan) for ventilation with room air at a tidal volume of 20 ml/kg at a rate of 18 breaths/min. To maintain a constant level of anesthesia, the dogs received continuous pentobarbital sodium (4 mg/kg/h). A thoracotomy was performed at the fifth left intercostal space, and the heart was suspended in a cardiac cradle. An arch vessel was cannulated with a 6-French cannula and the right femoral artery was perfused at 90 ml/min with the animal's own blood from the carotid artery using a Starling pneumatic resistor. Drugs were administered to the femoral artery via a catheter placed in the left femoral artery and connected to a pressure transducer (model MPU-0.5) for measurement of BP. Drugs were injected via a catheter placed in the left femoral vein. When BP had stabilized, the pressor response to i.v. administration of phenylephrine (10 µg/kg) was determined. In a preliminary study, the pressor response of phenylephrine was unchanged when repeatedly determined (seven determinations). Dogs were pretreated with increasing doses of OPC-28326 (1–1000 µg/kg), and the pressor responses were continuously recorded. In the case of prazosin, it was confirmed that the solvent (10% N,N-dimethylformamide), administered at a volume equal to that of 30 µg/kg drug, had no effect on the pressor response to phenylephrine. Prazosin was administered in a similar manner at 0.1 to 30 µg/kg, and the pressor response was recorded. The pressor responses induced by phenylephrine were expressed as percentages of those recorded before the first dose of OPC-28326 or prazosin was given.

Effects of OPC-28326 on FBF in Autoperfused Canine Femoral Artery Preparations

Dogs were injected with 700 U/kg heparin sodium i.v. after the induction of anesthesia. The right femoral artery was perfused at 90 ml/min with the animal's own blood from the carotid artery using a peristaltic pump (model 1219; Harvard Apparatus, Holliston, MA). A Starling pneumatic resistor was placed in parallel with the perfusion circuit to maintain the perfusion pressure at about 100 mm Hg. The blood flowing through the resistor was returned to the left femoral vein. Throughout the experiments, the animals were mechanically ventilated with room air via an endotracheal cannula connected to a respirator (model SN-480-3) using a tidal volume of 20 ml/kg at a rate of 18 breaths/min. The dogs were infused with pentobarbital sodium at 4 mg/kg/h to maintain anesthesia and with heparin sodium at 100 U/kg/h to prevent blood coagulation. FBF was measured with an electromagnetic flowmeter (MFV-2100) placed in the perfusion circuit. Drugs were administered to the femoral artery via a perfusion circuit using a microsyringe.

Cardiovascular Effects of OPC-28326 in Isolated, Blood-Perfused Canine Heart Preparations

Anesthetized dogs were injected with 500 U/kg heparin sodium i.v. and then exsanguinated. The heart was isolated, immersed in cooled lactate Ringer's solution, and used for various isolated heart experiments. These preparations were kept in liquid paraffin at 37°C in a glass container and perfused with arterial blood from the carotid...
artery of blood donor dogs through an arterial cannula using a peristaltic pump (model 1210; Harvard Apparatus). A Starling pneumatic resistor was placed in parallel with the perfusion circuit to maintain a constant perfusion pressure. The venous blood from the preparations and the blood passing through the resistor were collected in a blood reservoir and returned to the left jugular vein of the donor dog. The donor dogs were injected with 500 U/kg heparin sodium i.v. after the induction of anesthesia. Throughout the experiment, the dogs were ventilated and maintained with heparin and anesthesia as described above.

Sinoatrial node preparations, consisting of the right atrium, were prepared according to the method of Kubota and Hashimoto (1973). A cannula was inserted into the right coronary artery for perfusion at a constant pressure of about 100 mm Hg. Preparations were preloaded with a load of 1 g and stimulated with rectangular pulses (voltage, 1.2 times the threshold voltage) at a constant pressure of about 100 mm Hg. The CF of the right atrium was measured isometrically with a force displacement transducer (type 45496A; NEC Medical Systems) triggered by right atrial contraction. The right atrium preparations were preloaded with a load of 2 g. Drugs were administered using a microsyringe via a catheter connected to the right coronary artery.

Papillary muscle preparations were prepared from the ventricular septum and anterior chamber wall according to the method of Endoh and Hashimoto (1970). A cannula was inserted into the anterior septal artery (ASA) for perfusion at a constant pressure of about 100 mm Hg. Preparations were preloaded with a load of 1 g and stimulated with rectangular pulses (voltage, 1.2 times the threshold voltage; duration, 5 ms; frequency, 120 stim/min) generated by an electric stimulator (type 2907; NEC Medical Systems) applied through electrodes placed at the origin of the papillary muscle. The CF of the papillary muscle was measured isometrically. Blood flow of ASA was measured as an index of CBF. The drugs were administered using a microsyringe via a catheter connected to the ASA.

In the studies mentioned above, OPC-28326 at single doses of 0.1 to 100 nmol was administered when the pretreatment values of all the parameters had stabilized. When parameters returned to pretreatment levels after the administration of a single dose of the drug, another single dose was administered. To compare the potency in increasing FBF in autoperfused canine femoral artery preparations, OPC-28326 (0.1–100 nmol), prazosin (1–300 nmol), and yohimbine (1–300 nmol), a selective α2-adrenoceptor blocker (Shepperton et al., 1981), were administered via the femoral artery.

### Table 1

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Membrane Source</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine A1</td>
<td>[3H]DPCPX</td>
<td>Rat whole brain</td>
<td>5 %</td>
</tr>
<tr>
<td>Adenosine A2A</td>
<td>[3H]CGS-21680</td>
<td>Rat striatum</td>
<td>10 %</td>
</tr>
<tr>
<td>Adrenergic α1, nonselective</td>
<td>[3H]Prazosin</td>
<td>Rat whole brain</td>
<td>33 %</td>
</tr>
<tr>
<td>Adrenergic α2, nonselective</td>
<td>[3H]Rauwolscine</td>
<td>Rat cerebral cortex</td>
<td>95 %</td>
</tr>
<tr>
<td>Adrenergic β1</td>
<td>[3H]CGP-12177</td>
<td>Human recombinant</td>
<td>10 %</td>
</tr>
<tr>
<td>Adrenergic β2</td>
<td>[3H]CGP-12177</td>
<td>Human recombinant</td>
<td>1 %</td>
</tr>
<tr>
<td>Adrenergic β3</td>
<td>[3H]Iodocyanopindolol</td>
<td>Human recombinant</td>
<td>12 %</td>
</tr>
<tr>
<td>Angiotensin AT1</td>
<td>[3H]Losartan</td>
<td>Rabbit adrenal gland</td>
<td>9 %</td>
</tr>
<tr>
<td>Atrial natriuretic factor</td>
<td>[125I]Labeled ANF</td>
<td>Guinea pig adrenal gland</td>
<td>14 %</td>
</tr>
<tr>
<td>Bradykinin B1</td>
<td>[3H]des-Arg9-kallidin</td>
<td>Human HS 729 cell</td>
<td>20 %</td>
</tr>
<tr>
<td>Bradykinin B2</td>
<td>[3H]Bradykinin</td>
<td>Guinea pig ileum</td>
<td>5 %</td>
</tr>
<tr>
<td>Ca2+ channel (L)</td>
<td>[3H]Diltiazem</td>
<td>Rat cerebral cortex</td>
<td>19 %</td>
</tr>
<tr>
<td>Dopamine D1</td>
<td>[3H]SCH23390</td>
<td>Human recombinant</td>
<td>19 %</td>
</tr>
<tr>
<td>Endothelin ETα</td>
<td>[125I]Labeled endothelin</td>
<td>Rat A10 cell</td>
<td>11 %</td>
</tr>
<tr>
<td>Endothelin ETβ</td>
<td>[125I]-Labeled endothelin-1</td>
<td>Human recombinant</td>
<td>19 %</td>
</tr>
<tr>
<td>Histamine H1, peripheral</td>
<td>[3H]Pyrilamine</td>
<td>Guinea pig lung</td>
<td>5 %</td>
</tr>
<tr>
<td>Histamine H2</td>
<td>[3H]-[NH2]-potentidine</td>
<td>Guinea pig striatum</td>
<td>6 %</td>
</tr>
<tr>
<td>Muscarinic, nonselective</td>
<td>[3H]QNB</td>
<td>Rat cortex</td>
<td>19 %</td>
</tr>
<tr>
<td>Neuropeptide Y1</td>
<td>[3H]Labeled neuropeptide Y</td>
<td>Human SK-N-MC cell</td>
<td>11 %</td>
</tr>
<tr>
<td>Neuropeptide Y2</td>
<td>[3H]Labeled neuropeptide Y</td>
<td>Rabbit kidney medulla</td>
<td>0 %</td>
</tr>
<tr>
<td>Opiate, nonselective</td>
<td>[3H]Naloxone</td>
<td>Rat whole brain</td>
<td>3 %</td>
</tr>
<tr>
<td>Platelet-activating factor</td>
<td>[3H]PAF</td>
<td>Rabbit platelet</td>
<td>13 %</td>
</tr>
<tr>
<td>Purinergic P2X7</td>
<td>[3H]H-β-me-ATP</td>
<td>Rabbit urinary bladder</td>
<td>23 %</td>
</tr>
<tr>
<td>Serotonin 5-HT2</td>
<td>[3H]Ep Separacin</td>
<td>Rat whole brain</td>
<td>12 %</td>
</tr>
<tr>
<td>Thromboxane A2</td>
<td>[3H]SQ-22548</td>
<td>Rabbit platelet</td>
<td>12 %</td>
</tr>
<tr>
<td>Vasoactive intestine peptide VIP1</td>
<td>[3H]-Labeled VIP</td>
<td>Guinea pig lung</td>
<td>12 %</td>
</tr>
<tr>
<td>Vasoressin V1</td>
<td>[3H]Arg-Vasopressin</td>
<td>Rat liver</td>
<td>16 %</td>
</tr>
</tbody>
</table>

### Table 1.2

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate, Reaction</th>
<th>Source</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO synthase, constitutive</td>
<td>[3H]Arginine → [3H]citrulline + NO</td>
<td>Rat cerebellum</td>
<td>−8 %</td>
</tr>
<tr>
<td>Phosphodiesterase-III</td>
<td>[3H]cAMP → [3H]AMP (→ [3H]adenosine)</td>
<td>Human platelet</td>
<td>1 %</td>
</tr>
<tr>
<td>Phosphodiesterase-V</td>
<td>[3H]GMP → [3H]GMP (→ [3H]guanosine)</td>
<td>Human platelet</td>
<td>−6 %</td>
</tr>
<tr>
<td>Ca2+/calmodulin-dependent PK II</td>
<td>BB40 + [γ-32P]ATP → [32P]BB40 + ADP</td>
<td>Rat brain</td>
<td>−4 %</td>
</tr>
<tr>
<td>Protein kinase C, mixture of α, β, and γ</td>
<td>Histone H2 (H2) + [γ-32P]ATP → [32P]HH + ADP</td>
<td>Rat brain</td>
<td>−19 %</td>
</tr>
</tbody>
</table>
0.3 mM EDTA-Na$_2$O gassed with 95% O$_2$/5% CO$_2$, and stripped of connective and adipose tissues. The vessel was then cut into rings measuring 4 to 5 mm in length. Endothelial cells were carefully removed with forceps. The preparations were suspended vertically in an organ bath containing 20 ml of Krebs-Henseleit solution. The upper ends were connected to a force displacement transducer (UL-20GR; Minebea, Nagano, Japan). Initially, the vessel preparation was repeatedly contracted with 40 or 60 mM KCl. After the responses had stabilized, contraction was initiated with 5-HT (3 × 10$^{-7}$ M) or phenylephrine (3 × 10$^{-6}$ M). Concentrations were used that induced about 80% of maximum contraction. When contraction stabilized, OPC-28326 was cumulatively added to the organ bath at concentrations of 10$^{-8}$ to 10$^{-6}$ M. The vasorelaxing activities of OPC-28326 were expressed as percentages of contraction before treatment with the drug.

**α₂-Adrenoceptor-Blocking Property of OPC-28326 in Rat Perfused Hindquarters**

Rat perfused hindquarters were prepared according to a modification of the method of van Meel et al. (1983). Male Sprague-Dawley rats were treated with reserpine (5 mg/kg i.p.) and anesthetized with pentobarbital sodium 24 to 32 h later. The right hindquarter was perfused with modified Tyrode’s solution containing OPC-28326 (10$^{-8}$ to 10$^{-6}$ M) and 10$^{-7}$ M yohimbine or vehicle (distilled water) at constant pressure (55 cm H$_2$O) through the abdominal aorta. The rats were sacrificed by exsanguination, and the vena cava cœdaulis was cut to secure the outflow of perfusate. After stabilization, 1 ng to 30 μg of brimonidine, a selective α₂-adrenoceptor agonist (Guima-rases and Nunes, 1990; Thomas et al., 1994), was added to the perfusate in a volume of 10 μl. Changes in the perfusion flow rate of the hindquarter were expressed as percentages of the flow rate before the first dose of brimonidine.

Modified Tyrode’s solution was composed of 136.8 mM NaCl, 2.68 mM KCl, 0.26 mM MgCl$_2$, 0.42 mM NaH$_2$PO$_4$, 11.9 mM NaHCO$_3$, 1.8 mM CaCl$_2$, and 15 mM glucose, gassed with 95% O$_2$/5% CO$_2$, and maintained at room temperature (18–20°C).

**Drugs**

OPC-28326 (Otsuka Pharmaceutical Company, Tokyo, Japan), yohimbine (Sigma Chemical Co., St. Louis, MO), and phenylephrine (Wako Pure Chemical Industries, Osaka, Japan) were dissolved in distilled water. Atropine (Nacalai Tesque, Kyoto, Japan) and dibucaine hydrochloride (Wako) were dissolved in distilled water. Nadolol (Sigma Chemical Co.) was dissolved in 0.5 N HCl. Prazosin (Sigma Chemical Co.) and dibucaine hydrochloride (Wako) were dissolved in 0.5 N HCl. Prazosin was dissolved in modified Tyrode’s solution.

**Statistical Analysis**

In all experiments, values are expressed as mean ± S.E., and differences were considered statistically significant at P < .05.

**Cardiovascular Effects of OPC-28326 in Anesthetized Open-Chest Dogs.** Differences between pretreatment and post-treatment values were analyzed by the paired t test (two-tailed) at each dose. Myocardial CF was expressed as a percentage of the value before the administration of 0.1 μg/kg OPC-28326, and the differences between normalized pretreatment values and post-treatment peak values were analyzed by Student’s t test (two-tailed) at each dose.

**α₁-Adrenoceptor-Blocking Property of OPC-28326 in Spinally Anesthetized Dogs.** The difference between the basal value of systolic BP and that of the pressor response induced by i.v. injection of phenylephrine (10 μg/kg) for the OPC-28326- and prazosin-preadministration groups was analyzed by t test. The effects of the pretreatment with each drug were expressed as percentages of the control pressor response that was induced by phenylephrine. A parallel-line assay was performed using the dose-response curves of the drugs. When these dose-response curves showed parallelism, the potency ratio was calculated from their estimated equations.

**Effects of OPC-28326 on FBF in Canine Autoperfused Femoral Artery Preparations and on CBF, CF, and SR in Blood-Perfused Canine Heart Preparations.** Differences between pretreatment and post-treatment peak values were analyzed by the paired t test (two-tailed) at each dose.

**Results**

**Cardiovascular Effects of OPC-28326 in Anesthetized Open-Chest Dogs.** The basal blood values of FBF, VBF, CaBF, CFR, and MBF in seven dogs were 60.0 ± 9.3, 18.4 ± 2.0, 48.3 ± 7.3, 15.3 ± 1.8, 54.1 ± 6.5, and 72.7 ± 11.8 ml/min, respectively. The basal values of systolic and diastolic BPs and HR were 144 ± 6 mm Hg, 97 ± 4 mm Hg, and 169 ± 5 beats/min, respectively. Typical changes in hemodynamic parameters measured after single bolus injection of OPC-28326 are shown in Fig. 2. The effects of OPC-28326 on various artery flows and BP, HR, and myocardial CF are shown in Figs. 3 and 4, respectively. OPC-28326 increased FBF dose-dependently. Even at 0.3 μg/kg, FBF increased in all preparations and the increase was statistically significant. FBF increased by 7, 21, and 83% at a dose of 0.3, 1, and 30 μg/kg, respectively. VBF, CaBF, CFR, and MBF showed small biphasic changes: an increase followed by a decrease, or vice versa (Fig. 2). OPC-28326 at doses of ≤1 μg/kg had virtually no effect on VBF and RBF. Although MBF, CaBF, and CBF were increased significantly at doses of ≥0.3 or ≥1 μg/kg, these changes were very small, within 3, 1, and 1% of pretreatment values at a dose of 0.3 μg/kg, respectively, and within 5, 3, and 4% of pretreatment values at a dose of 1 μg/kg, respectively. Even at highest dose of 30 μg/kg, the changes were within 13, 9, and 20%, respectively (Fig. 3). OPC-28326 at doses of ≤1 μg/kg had virtually no effect on myocardial CF and systolic and diastolic BPs (Fig. 4). OPC-28326 increased HR significantly even at doses of 0.3 and 1 μg/kg, but the effect was very small, increasing by only 0.9 ± 0.3 and 2.6 ± 0.4 beats/min at each respective dose.

**Comparison of Effects of OPC-28326 and Prazosin on Cardiovascular System in Anesthetized Open-Chest Dogs.** Prazosin increased FBF dose-dependently. However, the increase was observed only at the doses that decreased BP (Fig. 5). On the other hand, OPC-28326 increased FBF dose-dependently, and the increase was statistically significant at all doses examined (Fig. 5). The systolic and diastolic...
BPs were significantly decreased at higher doses (10 and 30 \( \mu \)g/kg). Thus, OPC-28326 increased FBF without any effect on systolic and diastolic BPs at lower doses.

\[\text{\(a_1\)-Adrenoceptor-Blocking Property of OPC-28326 in Spinally Anesthetized Dogs.}\]

The baseline systolic BP values in the OPC-28326- and prazosin-treated groups before phenylephrine administration were 105 \( \pm \) 7 and 107 \( \pm \) 2 mm Hg, respectively. The increases in systolic BP after i.v. administration of phenylephrine (10 \( \mu \)g/kg) in the OPC-28326 and prazosin groups were 95 \( \pm \) 11 and 108 \( \pm \) 22 mm Hg, respectively. Baseline systolic BP and pressor responses induced by phenylephrine were not significantly different between the OPC-28326 and prazosin groups. OPC-28326 at doses of 1 to 10 \( \mu \)g/kg hardly affected systolic BP. OPC-28326 (30–1000 \( \mu \)g/kg) and 1 to 30 \( \mu \)g/kg prazosin dose-dependently inhibited the phenylephrine-induced increases in systolic BP (Fig. 6). When the doses of 30 to 1000 \( \mu \)g/kg OPC-28326 and 1 to 30 \( \mu \)g/kg prazosin were used, parallelism between these two compounds was found. Based on the regression lines yielding from the parallel assay, the potency of OPC-28326 in inhibiting the pressor response to phenylephrine was about 180 times less than that of prazosin (Fig. 6).

**Effects of OPC-28326 on FBF in Canine Autoperfused Femoral Artery Preparations and on CBF, CF,**

\[\text{\(a_1\)-Adrenoceptor-Blocking Property of OPC-28326 in Spinally Anesthetized Dogs.}\]

The baseline systolic BP values in the OPC-28326- and prazosin-treated groups before phenylephrine administration were 105 \( \pm \) 7 and 107 \( \pm \) 2 mm Hg, respectively. The increases in systolic BP after i.v. administration of phenylephrine (10 \( \mu \)g/kg) in the OPC-28326 and prazosin groups were 95 \( \pm \) 11 and 108 \( \pm \) 22 mm Hg, respectively. Baseline systolic BP and pressor responses induced by phenylephrine were not significantly different between the OPC-28326 and prazosin groups. OPC-28326 at doses of 1 to 10 \( \mu \)g/kg hardly affected systolic BP. OPC-28326 (30–1000 \( \mu \)g/kg) and 1 to 30 \( \mu \)g/kg prazosin dose-dependently inhibited the phenylephrine-induced increases in systolic BP (Fig. 6). When the doses of 30 to 1000 \( \mu \)g/kg OPC-28326 and 1 to 30 \( \mu \)g/kg prazosin were used, parallelism between these two compounds was found. Based on the regression lines yielding from the parallel assay, the potency of OPC-28326 in inhibiting the pressor response to phenylephrine was about 180 times less than that of prazosin (Fig. 6).
and SR in Blood-Perfused Canine Heart Preparations. FBF in six autoperfused femoral artery preparations was 27 ± 5 ml/min at a constant pressure of about 100 mm Hg. The basal tension developed in five papillary muscles stimulated at a rate of 120 stimuli/min was 6.8 ± 0.7 g, and the basal blood flow of ASA was 7.7 ± 1.0 ml/min at a constant pressure of about 100 mm Hg. In five sinoatrial node preparations, the basal SR was 98 ± 6 beats/min. Figure 7 shows the effects of OPC-28326 (0.1–100 nmol) on FBF in the constant pressure autoperfused canine femoral artery preparations and on CBF, CF of papillary muscles, and SR in isolated, blood-perfused canine heart preparations. In this series of experiments, repeated intra-arterial administration of OPC-28326 did not affect the pretreatment values (data not shown). OPC-28326 dose-dependently increased FBF, with a 37% increase at a dose of 100 nmol in the constant-pressure autoperfused femoral artery preparations (Fig. 7). The increase in FBF was significant at doses of ≥30 nmol. OPC-28326 increased FBF at doses of 3 and 10 nmol in all five preparations, although these were not significant. In the isolated blood-perfused heart preparations, OPC-28326 at the doses tested had no effect on CBF, CF of papillary muscles, or SR (Fig. 7).

Effects of OPC-28326, Prazosin, and Yohimbine on FBF in Autoperfused Canine Femoral Artery Preparations. Baseline values of FBF in the OPC-28326-, prazosin-, and yohimbine-administered groups were 49.0 ± 8.5, 40.1 ± 9.3, and 51.0 ± 6.4 ml/min, respectively. There were no statistically significant differences among the drug-administered groups. OPC-28326, prazosin, and yohimbine increased FBF dose-dependently at doses of ≥1, ≥10, and ≥10 nmol, respectively (Fig. 8). Statistical analysis revealed parallelism between OPC-28326 (1–10 nmol), prazosin (10–100 nmol), and yohimbine (1–10 nmol). It was found from the line assay that yohimbine was approximately equipotent with OPC-28326 and that prazosin was 14 times less potent than OPC-28326.

Receptor and Enzyme Studies. The results of OPC-28326 on various receptors and enzymes are listed in Table 1. Effects of intra-arterial OPC-28326 on FBF, CBF, SR, and papillary muscle CF in constant-pressure autoperfused canine femoral artery and isolated blood-perfused canine heart preparations. Each point is mean ± S.E. of five constant-pressure autoperfused canine femoral artery or six isolated blood-perfused canine heart preparations. *P < .05 compared with pretreatment values (paired t test).

Even at 10 μM, the inhibitory effect of OPC-28326 on the enzyme activities listed and on the receptor binding did not reach 50% except for α2-adrenoceptors (Table 1). OPC-28326, at a concentration of 10 μM, inhibited the specific binding of [3H]rauwolscine (10 μM), a preferential α2-adrenoceptor blocker (Tanaka et al., 1978), by 95%. Further binding assay revealed that OPC-28326 inhibited the specific binding of [3H]rauwolscine to a rat brain preparation in a concentration-dependent manner (10 nM to 3 μM). The Ki value for α2-adrenoceptor was 337 ± 81 nM.

Inhibitory Action of OPC-28326 against Contraction Induced by 5-HT and Phenylephrine in Isolated Femoral Artery Preparations. Isolated canine femoral artery ring preparations were contracted with 3 × 10−7 M 5-HT or 3 × 10−6 M phenylephrine. Tension developed for each group was 3.66 ± 0.40 g and 4.75 ± 0.38 g, respectively. OPC-28326 relaxed phenylephrine-induced contraction concentration-dependently. It did not, however, relax 5-HT-induced contraction until 10−5 M was reached (Fig. 9).

α2-Adrenoceptor-Blocking Property of OPC-28326 in Rat Perfused Hindquarters. In six perfused rat hindquarters, pretreatment values for perfusion of the modified Tyrode’s solution with OPC-28326 at concentrations of 10−8, 10−7, and 10−6 M, yohimbine at a concentration of 10−7 M, and its vehicle were 8.17 ± 0.44, 7.80 ± 0.52, 8.10 ± 0.48, 8.28 ± 0.5, and 7.88 ± 0.58 ml/min, respectively, at a constant pressure of about 55 cm H2O. These values are not significantly different among the examined groups.
brimonidine (1 ng to 30 μg) was injected into the perfusate, perfusion flow was reduced in a concentration-dependent manner (Fig. 10). OPC-28326 at concentrations of 10^{-7} and 10^{-6} M inhibited flow reduction in a concentration-dependent manner (Fig. 10). Yohimbine, at a dose of 10^{-7} M, inhibited brimonidine-induced flow reduction significantly (Fig. 10). Exploratory analysis revealed that the inhibitory action of OPC-28326 at a concentration of 10^{-6} M was not significantly different from that of yohimbine at a concentration of 10^{-7} M, suggesting that OPC-28326 is at least 10 times less potent than yohimbine in inhibiting α2-adrenoceptor activity.

Discussion

In the present study, OPC-28326 increased FBF dose-dependently with little change in systolic BP, diastolic BP, myocardial CF, and HR in anesthetized open-chest dogs. The blood flow to other organs, such as CBF, CaBF, VBF, RBF, and MBF, showed biphasic changes with an increase followed by a decrease, or vice versa, in the same preparations. The changes in these organ flows were smaller than that of FBF, especially at lower doses. OPC-28326 increased FBF dose-dependently in autoperfused canine femoral artery preparations when the drug was injected directly into femoral artery. The drug had no effect on HR, CF of papillary muscles, and CBF in isolated, blood-perfused canine heart preparations when injected directly into the coronary artery. From these results, we suggest that OPC-28326 is a dilator of femoral beds with little or no effect on other cardiovascular parameters.

A selective increase in blood flow to the hindlimb may be beneficial to patients with peripheral arterial insufficiency of the leg. There is, however, a controversy about the efficacy of vasodilators for this condition. Coffman (1979) reviewed past clinical trials and concluded that drugs that exerted vasodilation by affecting the sympathetic nervous system or via direct action were without value for patients with peripheral occlusive arterial disease (POAD). There are, however, some vasodilators that have been clinically proved to ameliorate POAD. Naftidrofuryl, an antagonist of 5-HT2 receptors, for example, caused a vasodilation in the legs of dogs and humans (Barradell and Brogden, 1996). This agent increased pain-free walking distance in patients with intermittent claudication caused by POAD to a greater extent than placebo (Barradell and Brogden, 1996). Cilostazol, a phosphodiesterase-3 inhibitor, caused a flow increase during reactive hyperemia in the lower extremities of patients with arteriosclerosis obliterans (Yasuda et al., 1985), and this drug has beneficial effects in the treatment of intermittent claudication (Dawson et al., 1998). Buflomedil increased FBF dose-dependently in dogs (Vanhoutte, 1984) and improved POAD in humans (Clissold et al., 1987). Although these agents have other interesting effects, such as inhibition of platelet aggregation and/or favorable rheological actions (Clissold et al., 1987; Okuda et al., 1993; Barradell and Brogden, 1996), their common pharmacological effect, vasodilation, probably contributes in a major way to the amelioration of POAD. Roberts et al. (1987) reported that β-adrenoceptor blockers decreased pain-free and maximum walking distances on a treadmill at doses that reduced blood pressure in patients with hypertension complicated by intermittent claudication. Reduced systemic arterial pressure and reduction in cardiac output by a drug might exacerbate the reduction in perfusion pressure to the lower limb. Decreases in perfusion pressure can reduce lower limb blood flow as a consequence of compensatory adrenoceptor-mediated vasoconstriction in the collateral circulation and possibly in the stenotic vessel (Roberts et al., 1987). From these points of view, the selective and direct increase in FBF by OPC-28326 provides a good possibility for amelioration of POAD.

One of the possible mechanisms of vasodilation is the increase in cyclic nucleotides, such as cAMP and cGMP (Murray, 1990). Indeed, cilostazol and zaprinast, an inhibitor of phosphodiesterase-5, which cause increases in cAMP and cGMP, respectively, induced vasodilation (Kamiya and Sakaguchi, 1985; Trapani et al., 1991). OPC-28326, however, had almost no effect on these phosphodiesterases. It has been reported that postsynaptic α-adrenoceptors exist in the hindlimb vasculature in dogs (Langer et al., 1981) and in rats (van Meel et al., 1983). Satoh et al. (1985) reported that an α1-adrenoceptor antagonist preferentially increased FBF in anesthetized dogs. Binding studies indicate that OPC-28326 has a reasonable affinity to α1-adrenoceptors. In this study, an inhibitory action of the phenylephrine-induced pressor response by OPC-28326 in spinally anesthetized dogs was, however, about 180 times less potent than that of prazosin, suggesting that OPC-28326 possesses α1-adrenoceptor antagonistic activity, but its potency is very weak compared with prazosin. On the other hand, the potency in increasing FBF by OPC-28326 is 14 times more potent than that of prazosin. FBF was increased by OPC-28326 at doses that caused no effect on BP in open-chest dogs. Prazosin did increase FBF at doses that caused a decrease in BP. In other words, prazosin did not selectively increase FBF at any dose examined. Thus, we conclude that the α1-adrenoceptor blockade per se is not the main mechanism of the FBF-increasing action of OPC-28326.

Receptor binding studies showed that OPC-28326 has an affinity to 5-HT2 receptors. It has been reported that a bolus injection of 5-HT in rat produced an increase in perfusion pressure in rat hindquarters (Verheyen et al., 1991). In collateralized femoral vascular beds, 5-HT-induced decreases in hindlimb flow was enhanced and the decrease was reduced by 5-HT2 receptor antagonists (Orlandi et al., 1986; Verheyen et al.,
ing studies revealed that OPC-28326 has a high affinity to brimonidine-induced contraction. Furthermore, OPC-28326 inhibited brimonidine-induced increased FBF in autoperfused canine femoral artery preparations. The inhibitory action of OPC-28326 against brimonidine-induced contraction was, if anything, much weaker than that of yohimbine. This suggests that the $\alpha_2$-adrenoceptor may, at least in part, contribute to the contractile regulation of the femoral vascular bed (Horn et al., 1982). In the latter study, yohimbine increased FBF in autoperfused canine femoral artery preparations and inhibited the perfusion flow decrease in the hindlimb induced by brimonidine. These data suggest that $\alpha_2$-adrenoceptor antagonists may increase FBF. OPC-28326 increased FBF in autoperfused canine femoral artery preparations. Furthermore, OPC-28326 inhibited brimonidine-induced decrease in perfusion flow in rat perfused hindquarters. Binding studies revealed that OPC-28326 has a high affinity to $\alpha_2$-adrenoceptors ($K_i = 337 \pm 81$ nM). Thus, $\alpha_2$-adrenoceptor antagonistic action may be one of the important mechanisms of action of OPC-28326.

The inhibitory action of OPC-28326 against brimonidine-induced flow reduction was, however, at least 10 times less potent than that of yohimbine in rat perfused hindquarters. On the other hand, the FBF-increasing effect of OPC-28326 is almost as potent as that of yohimbine in autoperfused canine femoral artery preparations. Thus, it is clear that some unknown mechanisms may contribute to the increase in blood flow. Further studies are required to reveal the exact mechanisms of selective vasodilation.

In conclusion, low doses of OPC-28326 increased FBF in anesthetized open-chest dogs with little changes in BP, HR, CF, and blood flow in other arteries, such as coronary, carotid, vertebral, renal, and mesenteric arteries. We suggest that this new drug is a selective peripheral vasodilator and may be of clinical relevance in some peripheral vascular disorders.

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